Exhibit E

1: Prog Clin Biol Res. 1987;231:443-57.

Links

Recovery of endotoxin from human plasma by acid oxidative treatments as monitored by an automated microtiter plate-chromogenic substrate Limulus amebocyte lysate (LAL) assay method.

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Methods to detect endotoxin in human plasma for LAL testing have been developed by use of a perchloric acid (PCA) or a trifluoroacetic acid (TFA) treatment. These acid oxidative treatments eliminate interferences present in plasma. The mode of action of the acids for the elimination of the interferences is indicated to be by their oxidative actions. The treated plasma is assayed by use of an automated microtiter plate-chromogenic substrate LAL assay method employing a modified Cetus Pro/Pette EXPRESS system. The automated system assays 96 samples in approximately 22 minutes. Assay sensitivity of the automated Pro/Pette system is approximately 0.01 endotoxin units (EU) per ml with a linear range from 0.03 to 0.14 EU/ml. The relative standard deviation (RSD) of the assay system is approximately 6%. The improved precision of the LAL assay by the automated system has been attributed to its fast speed (2 minutes) in dispensing the unstable LAL reagent to all 96 samples in one assay block. The total operation, including the acid oxidative treatments and the 96 sample LAL assay, takes approximately 45 minutes. The recovery of spiked endotoxin from plasma ranged from 75 to approximately 100% by adding 0.4 ml of either 0.1-0.2 N PCA or 0.2-0.35 N TFA to 0.2 ml plasma and reacting for 15 minutes at 37 degrees C. The standard deviation of the assay is approximately 0.1 EU/ml plasma. The method has been proven to be rugged, simple, rapid, and cost effective thus would be suitable for clinical application.

PMID: 3035582 [PubMed - indexed for MEDLINE]